Partition Coefficients of Some Antioxidants in Butteroil-Water Model Systems

D. G. CORNELL, E. D. DeVILBISS, and M. J. PALLANSCH
Dairy Products Laboratory
Eastern Utilization Research and Development Division[1]
Washington, D.C. 20250

Abstract

The butteroil/water partition coefficients of the esters of gallic acid, butylated hydroxyanisole, and nordihydroguaiaretic acid have been determined. Butylated hydroxyanisole exhibited the highest preference for the oil phase, with a partition coefficient over 800. The coefficients of the gallic acid esters increased with increasing chain length of the alcoholic substituent, ranging from 0.24 for ethyl gallate to 71 for hexyl gallate. Increasing the temperature lowered the coefficient for the gallates, with the effect most pronounced for the higher homologues. Milk salts exhibited no effect on the coefficient of nordihydroguaiaretic acid or butylated hydroxyanisole but caused a decrease of partitioning of the gallates.

Introduction

Stabilization of the initial flavor of whole milk powders produced by various spray (3) and vacuum (6) techniques is one of the objectives of the research carried out in the Dairy Products Laboratory. Enhanced flavor stability of these powders has been demonstrated with inert gas (9) or heat treatment of the milk before drying (7). Objections arise to the economics of the inert gas packaging and to the cooked flavor developed in powders made from milk heated in excess of pasteurization requirements. A more satisfactory solution to such problems could be obtained if the successful protection of whole milk powders could be achieved with antioxidant additives. Although much previous work has been done with antioxidants in dry milk products (8), success has been limited, especially when comparing the results obtained with the same antioxidants added to other foods. Milk is a multiphase, multicomponent food, and one cannot assume that antioxidant added will automatically reside in the fat phase. The actual distribution of antioxidants in such a system is unknown. Indeed, analytical procedures on heterogeneous systems are either unreliable or tedious at best.

Our work was with model systems of bulk butteroil and water phases to determine the relative oil-water preference of the esters of gallic acid, butylated hydroxyanisole, and nordihydroguaiaretic acid. In addition, carbon 14–labeled propyl gallate was used to provide an independent check on the results obtained with the more usual photometric techniques.

Materials and Methods

Butteroil was prepared from fresh milk from a herd of Holstein cows maintained at Beltsville, Maryland. Propyl gallate was obtained from the J. T. Baker Company.[2] The butylated hydroxyanisole (BHA) was purchased from Nutritional Biochemicals Corporation[2] and the nordihydroguaiaretic acid (NDGA) from the Nordigard Corporation of Chicago, Illinois.[2] The ethyl, isopropyl, butyl, amyl, and hexyl gallates were synthesized according to the procedure of Russell and Tebbens (5). The gallic acid and ethyl through amyl alcohols were reagent-grade chemicals from the Fisher Scientific Company.[2] The hexyl alcohol was Eastman practical grade. Analysis on a Carbowax[2] GLC column indicated that it consisted of about 90% normal hexanol, with a second unidentified component comprising most of the remainder. However, the resulting hexyl gallate, after several recrystallizations from distilled water, gave a melting point of 92 C, in good agreement with literature values. The labeled propyl gallate was synthesized by the aforementioned technique with carbon 14–labeled propanol obtained from Mallinckrodt Chemical Company.[2] The salt solution used to simulate milk ultrafiltrate has been described by Jenness and Koops (4).

For the determination of partition coefficient, measured amounts of butteroil and anti-

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[2] Mention of brand or firm names does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

oxidant solutions were put into constant-temperature vessels under an atmosphere of nitrogen and equilibrated for one to three days with gentle stirring. The concentration of the antioxidant in the aqueous phase was then determined by the absorption of ultraviolet light or by liquid scintillation counting. For the absorbance measurements a Beckman model DU spectrophotometer was used, with the wavelength set at 270 mμ for the gallates, 286 mμ for BHA, and 283 mμ for NDGA. These corresponded to the wave lengths of maximum absorption as determined with a Perkin-Elmer Model 350 spectrophotometer. A 1-cm cell path was used for all analyses. Carbon 14–labeled propyl gallate was analyzed by liquid scintillation techniques with a Packard Model 3001 Tri-Carb liquid scintillation spectrometer. The solutions for counting were comprised of 6 ml of toluene containing 5 g/liter of 2,5-diphenyloxozole (PPO) and 0.3 g/liter of 1,4-bis-2-(methyl-5-phenyloxazolyl) benzene (dimethyl POPOP), 3.8 ml of absolute ethanol, and 0.2 ml of the aqueous or oil sample containing the labeled propyl gallate. Standard solutions were prepared from aqueous solutions of the labeled gallate. The PPO and dimethyl POPOP samples were purchased from the Packard Instrument Company.[2]

The partition coefficient of a compound, as determined in this work, is defined by the expression,

$$P_{o/w} = C_o/C_w \qquad [1]$$

where C_o and C_w are the equilibrium concentrations of the antioxidant in the oil and water phases, respectively. Direct determination of the antioxidant concentration in the oil and water phases was performed only with the labeled propyl gallate. Although techniques exist for the photometric analyses of antioxidants in oil phases (1), the authors found it more convenient to analyze the aqueous phase before and after partitioning had been effected. For a system in which the volumes of the oil and water phases are known, and where the concentration of antioxidant in the aqueous phase has been determined before and after partitioning, it can be shown that the partition coefficient is given by the expression,

$$P_{o/w} = V_w/V_o\ [C_s/C_f - 1] \qquad [2]$$

where V_w = volume of the water phase
V_o = volume of the oil phase
C_s = concentration of the starting aqueous antioxidant solution
C_f = concentration of the final aqueous antoxidant solution

If, for the analyses, identical dilutions of the starting and final antioxidant solutions are made, the absorbance or counts can be substituted for the appropriate concentrations in this expression.

Results

The effect of chain length and temperature on the oil/water partition coefficient of the alkyl gallates is shown in Figure 1. Each increase in the chain length by a methylene group effected about a three- to fivefold increase in the partition coefficient, with the effect decreasing toward the longer chain lengths. Increasing the temperature decreased the partition coefficients, with the effect becoming more pronounced for the higher homologues. The effect of a salt solution on the partition coefficient of the antioxidants is presented in Table 1. The salt solution, 0.6% in solids and buffered at a pH of 6.5, was designed to simulate the salts in milk. In most cases, this salt solution resulted in a lowering of the partition coefficient below that found with unbuffered water solutions. The direct analysis of the oil and water phases for carbon 14–labeled propyl gallate gave the same results as with photometric analysis of the water phase and Formula [2]. The partition coefficient of propyl gallate remained constant in acid solution to about pH 5.5. Further increase in pH effected a marked reduction in the partition coefficient (Fig. 2). Varying the ionic strength of these solutions over the

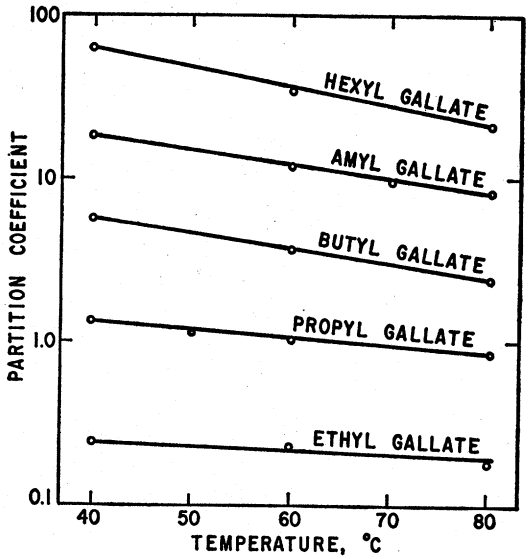

FIG. 1. Partition coefficients of some alkyl gallates.

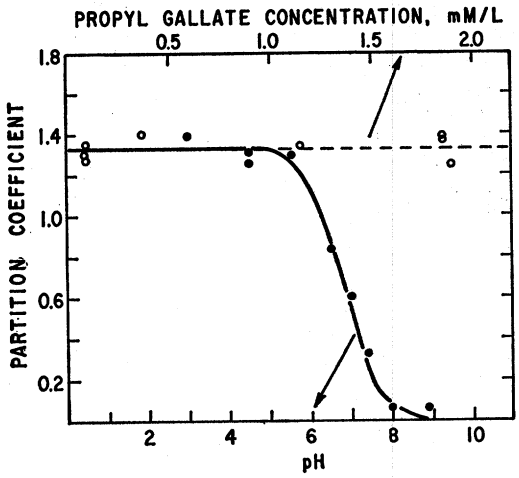

FIG. 2. Effect of pH (filled circles) and concentration (open circles) on the partition coefficient of propyl gallate.

range of 0.2 to 0.05 M effected no change in their partition coefficients. Figure 2 also shows that the partition coefficient of propyl gallate in oil/water systems is independent of the total gallate concentration. The same behavior has been noted for amyl gallate and BHA in oil/water systems and for propyl gallate in oil/salt solution systems.

Butylated hydroxytoluene (BHT) was too insoluble in the aqueous phase to be studied by the methods used here, although the analytical techniques were capable of detecting concentrations on the order of micromoles per liter.

Discussion

Several batches of butteroil made from Holstein herd milk over many seasons were used. The partition coefficient of propyl gallate showed no difference between batches. No attempt was made to determine the effect of breed on the antioxidant partition coefficients. The mean deviations in Table 1 were computed from three, or usually more, determinations of the partition coefficients. Initially, difficulty was experienced in obtaining values on the antioxidants containing the salt solution, due to the formation of a cloudy precipitate. This was probably owing to the precipitation of calcium phosphate in the warm solutions. The solutions were clarified for analysis by the addition of a drop of hydrochloric acid, which had no effect on the absorbance of the solutions other than to dissolve the precipitate.

The change in the partition coefficient as a function of the gallate chain length and the temperature of the system (Fig. 1) reflects the solubility behavior of these compounds. The partition coefficient of a compound is approximated by the ratio of its solubilities in the respective phases. Thus, we notice the expected increasing preference of the longer chain gallates for the oil phase. Increasing the temperature increases the preference of these compounds for the aqueous phase. The influence of the salt solution on the partition coefficients (Table 1) is probably due to the effect of pH. The influence of pH on the partition coefficient of propyl gallate can be accounted for by simple acid-base considerations. The phenolic hydrogens of propyl gallate will ionize in sufficiently basic solutions to yield gallate ions which presumably will not dissolve in the oil phase. The aqueous phase then contains both ionized and un-ionized gallates in amounts that can be determined, provided the ionization constant of the active hydrogen is known. The partition law [Formula (1)] will still apply for the un-ionized species; hence, the actual distribution of gallate can be calculated as a function of pH. This procedure was used to calculate the solid curve in Figure 2 with the following provisions: Since the pK's of the

TABLE 1. Buteroil/water partition coefficient of antioxidants at 40 C.

Antioxidant	Water	Milk salt solution
Ethyl gallate	0.24 (0.03)[a]	—
Propyl gallate	1.33 (0.05)[b]	0.84 (0.13)[a]
Isopropyl gallate	0.64 (0.02)[a]	—
Butyl gallate	5.8 (0.5)[a]	3.9 (0.1)[a]
Amyl gallate	18.5 (0.2)[a]	9.5 (0.2)[a]
Hexyl gallate	71.0 (1.1)[a]	44.8 (0.7)[a]
Nordihydroguaiaretic acid	21.7 (0.9)[a]	21.5 (1.1)[a]
Butylated hydroxyanisole	834 (4)[a]	825 (14)[a]

[a] Numbers in parentheses are mean deviations of three or more determinations.
[b] Standard deviation of 11 determinations.

phenolic hydrogens of propyl gallate are unknown, 6.8 was chosen for pK of the first hydrogen to provide the best fit with the experimental data. Further, to simplify the calculations, it was assumed that only ionization of the first hydrogen was important, with the two others being effectively suppressed over the range of pH's studied. Salt-free water in equilibrium with the atmosphere usually has a pH of about 5.5, due to dissolved CO_2. Hence, both the water and acid buffer solutions would tend to exhibit a suppressing effect on the ionization of the phenolic hydrogens of the gallates as compared to the more basic salt used in the systems reported in Table 1.

The independence of the partition coefficient on the total gallate concentration indicates that the simple ratio (Formula 1) is sufficient to express the distribution of these antioxidants between oil and water phases. For unbuffered systems such as antioxidant-water this also indicates that but a single species is present and undergoing partitioning. For a more complete discussion of the partition law, the reader is encouraged to consult Reference 2 or any text on liquid-liquid extraction.

The partition coefficients reported here should indicate in a relative way the amount of the corresponding antioxidant to be found in the fat phase of dry milk powders. The actual fraction of an antioxidant in the fat phase will also depend on additional factors. Assuming that partition equilibrium has been reached while the milk is still in a fluid state, the fraction of antioxidant in the fat will depend on the relative volumes of fat and water. For a concentrate of 50% total solids where the oil/water ratio is about 1:4, it can be shown that, on the basis of partitioning alone, the fat phase will contain about 20, 85, 90, and 99% of the total propyl gallate, NDGA, hexyl gallate, and BHA, respectively. The possible interaction of these antioxidants with the proteins of milk has been ignored in these calculations, however. Any such binding would tend to lower the amount of antioxidant in the fat phase, since partition equilibrium is established between the antioxidant in the oil phase and the free antioxidant in the aqueous phase. Presumably, the distribution established just before spray drying would be fixed in the powder by the rapid dehydration process. Thus, it can be seen that a knowledge of all of the interactions of antioxidants with the components of milk is necessary for the prediction of their distribution in dry powders.

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